Ongoing graft-*versus*-host disease is a risk factor for azoospermia after allogeneic hematopoietic stem cell transplantation: a survey of the Late Effects Working Party of the European Group for Blood and Marrow Transplantation

Alicia Rovó,[1] Mahmoud Aljurf,[2] Sandra Chiodi,[3] Simonetta Spinelli,[3] Nina Salooja,[4] Gülsan Sucak,[5] Ann Hunter,[6] Tan Swee Kim,[7] Gérard Socié,[8] Maria Teresa van Lint,[3] Jakob R. Passweg,[9] Mutlu Arat,[10] Manuela Badoglio,[11] and André Tichelli[1] on behalf of the Late Effects Working Party of the EBMT

[1]University Hospital of Basel, Basel, Switzerland; [2]King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia; [3]Ospedale San Martino, Genova, Italy; [4]Hammersmith Hospital, London, UK; [5]Gazi Universitesi Tip Fakültesi Hastanesi, Ankara, Turkey; [6]University Hospitals of Leicester NHS Trust, Leicester, UK; [7]Hospital Amphang Jalan Mewah Utara Pandan, Kuala Lumpur, Malaysia; [8]Paris VII Hospital Saint Louis, Paris, France; [9]Hôpitaux Universitaires de Genève, Switzerland; [10]Florence Nightingale Sisli Hospital, Istanbul, Turkey; and [11]EBMT Study Office, AP-HP, UPMC Univ Paris, France

ABSTRACT

The aim of this study was to assess the degree of spermatogenesis defects in sperm analysis in long-term male survivors after allogeneic hematopoietic stem cell transplantation in order to identify the risk factors related to potential infertility after hematopoietic stem cell transplantation and to provide data on longitudinal sperm recovery after hematopoietic stem cell transplantation. Here, the Late Effects Working Party of the European Group for Blood and Marrow Transplantation reports data of sperm analysis from 224 males who underwent hematopoietic stem cell transplantation. Median time between transplantation and sperm analysis was 63 months (8-275 months). At last sperm analysis, presence of any degree of spermatozoa was reported in 70 (31%) and complete azoospermia in 154 (69%) patients. In multivariate analysis, being conditioned with total body irradiation (RR 7.1; 95% CI: 3.4-14.8) and age over 25 years at transplantation (RR 2.4; 95% CI: 1.09-5.2) were significantly associated with higher risk for azoospermia. In patients not conditioned with total body irradiation, ongoing chronic graft-*versus*-host disease is the main adverse factor for sperm recovery (RR of 3.11; 95% CI: 1.02-9.47; *P*=0.045). Already established risk factors, such as total body irradiation and age older than 25 years at hematopoietic stem cell transplantation, were seen to be the most relevant adverse risk factor for sperm production after hematopoietic stem cell transplantation. Furthermore, for the first time, ongoing graft-*versus*-host disease has been shown to be the most relevant adverse factor for sperm recovery, particularly in patients conditioned without total body irradiation. We also introduce a useful scoring system to predict the probability of male long-term survivors' azoospermia.

Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) has become the treatment of choice for defined malignant and non-malignant hematologic disorders.[1] Given the growing number of transplants performed each year and the constantly improving outcomes over the last decades, the number of long-term survivors is steadily increasing.[2,3] General health status, the development of late events related to HSCT, and quality of life have become issues of great concern. Long-term survivors expect to recover their initial health status after HSCT and to lead a normal life with appropriate physical and psycho-social functioning.[4-6]

In long-term survivors, fertility preservation represents an important issue since gonadal dysfunction with absence of sperm production is a common finding in male patients receiving chemotherapy and irradiation prior to HSCT and as pre-transplantation conditioning.[7-12] Total body irradiation (TBI) used as part of the conditioning regimen has been shown to play a central role in subsequent infertility. However, with increasing follow-up time, even when conditioned with standard dose TBI, male recipients surviving more than ten years, under the age of 25 years at HSCT have some hope of recovery of spermatogenesis.[13,14] Because of the strong impact of TBI, other potential risk factors for long-term infertility may remain hidden. Particularly, it is not clear whether graft-*versus*-host disease (GVHD) impacts on sperm recovery of long-term survivors.[13] So far, predictors for male infertility after HSCT have not been well established.

Therefore, we conducted a retrospective multicenter study on sperm analysis (SFA) of a large cohort of male patients after allogeneic HSCT. All recipients' data were reported to the Late Effects Working Party (LEWP) of the European Group for Blood and Marrow Transplantation (EBMT). The aim of this study was to assess the degree of spermatogenesis defects in SFA in long-term male survivors after allogeneic

 Haematologica 2013;98. doi:10.3324/haematol.2012.071944
The online version of this article has a Supplementary Appendix.
Manuscript received on June 11, 2012. Manuscript accepted on August 24, 2012.
Correspondence: RovoA@uhbs.ch

HSCT, to identify the risk factors related to potential infertility after HSCT and to provide data on longitudinal sperm recovery after HSCT.

Design and Methods

For this retrospective multicenter study of the Late Effects Working Party of the EBMT all centers were asked if they had performed seminal analysis (SFA) in male patients before and after allogeneic HSCT, and if they would agree to provide information on all patients who had. Five hundred and forty-three centers were contacted of which 93 responded. Twenty-three Transplant Centers reported having data on SFA, and 19 of them finally contributed reports on a total of 259 patients; 224 of 259 were treated with allogeneic HSCT. Overall, 224 patients were included in this study.

The EBMT is a voluntary group of transplant centers each of which is required to provide transplant-related information on each patient using a specific anonymous data collection form. Patients provide written informed consent to have their data on disease, treatment and outcome, including late complications, reported to the registry. Clinical surveillance of HSCT recipients was approved by the local institutional review boards. Patients' characteristics, HSCT conditioning regimens and clinical outcome data were collected prospectively and stored in the EBMT database.

Details requested on seminal fluid parameters included the number and date of collections performed per patient, the sperm concentration, the motility and the morphology of the spermatozoa. Gonodotropic hormone and testosterone levels (if performed) were requested. Results of SFA were assessed according to the World Health Organization (WHO) guidelines.[15] Patients were considered to be normozoospermic when the sperm concentration exceeded $20x10^6$/mL, oligozoospermic when the sperm count was between 5 and $20x10^6$/mL, severely oligozoospermic with a sperm count below $5x10^6$/mL, and cryptospermic when spermatozoa were detected only after careful analysis of the concentrated sample. When no spermatozoa were detected patients were considered azoospermic.

Statistical analysis

Variables significantly associated with the risk of infertility after allogeneic HSCT were assessed by univariate and multivariate analysis. Any presence of spermatozoa in SFA was considered as existence of spermatogenesis. Patients with sperm detectable in the SFA were compared to patients with no evidence of spermatogenesis, using the χ^2 test for categorical data and the Mann-Whitney U test for continuous variables. Variables considered for infertility risk analysis were age at HSCT, disease, type of conditioning regimen (TBI≥7.5 Gray *vs.* busulfan containing regimen *vs.* regimen without TBI and without busulfan), occurrence of acute and chronic GVHD, persistence of chronic GVHD at time of SFA, continuous treatment with immunosuppression, and time interval between HSCT and SFA. For multivariate analysis, logistical regression with 2-sided significance levels was used to assess the impact of risk factors with infertility. A backward stepwise procedure was used to eliminate non-significant variables. Since conditioning with TBI presented the strongest impact on the risk of infertility after HSCT, a multivariate analysis including and not-including TBI as a variable was performed. Furthermore, a separate subgroup analysis was performed on patients with and without TBI.

To predict infertility risk after allogeneic HSCT a score system was set up and applied to all patients. Significant variables in univariate analysis were included into the score calculation and weighted according to their impact. As a result, TBI counted for 2 points, age over 25 years at HSCT and ongoing chronic GVHD at time of SFA for 1 point each, and time interval between HSCT and SFA under eight years for 0.5 point. Patients with a score of 0 to 1.0 point were considered as low-risk, those with a score of 1.5 to 3 points as intermediate-risk, and those with a score of 3.5 points or more as high-risk for presenting infertility after allogeneic HSCT. We included in this calculation only patients where all 4 variables were available.

Finally, subgroup analysis was performed on male patients for whom results on SFA before and after HSCT were available to assess the role of pre-existing spermatogenesis defects, and in those patients in which more than one SFA after allogeneic HSCT was collected, the longitudinal recovery spermatogenesis capacity was evaluated. In all statistical procedures, $P<0.05$ was considered as the level of significance. Statistical analysis was performed using SPSS statistical software (IBM SPSS Statistics, version 19, IBM Co.).

Patients' characteristics

From the 224 patients included in the present study, 166 (74%) had data on one SFA and 58 (26%) on two or more SFA after allogeneic HSCT. Data on SFA collected before and after HSCT were available from 17 (8%) patients. For the longitudinal subgroup analysis on 58 patients who had more than one SFA post-transplant, data of the first and last SFA collected after HSCT were compared. Characteristics of these 224 patients are presented in Table 1. Most of the patients were under 40 years of age at time of HSCT (210 of 224; 94%). The participating centers and the number of patients provided by each center are listed in the Appendix.

Results

Results of seminal fluid analysis

In the last SFA, presence of any degree of spermatozoa was reported in 70 (31%), and complete azoospermia in 154 (69%) patients. Among those with spermatogenesis, 22 (10%) patients had normozoospermia, 13 (6%) oligozoospermia, 28 (13%) severe oligozoospermia and 7 (3%) cryptospermia. Details on SFA, including data on sperm concentration, motility and morphology are shown in Table 2. Additional data on hormonal status (FSH, LH and testosterone) at SFA time are also provided in the same table.

Risk factors analysis associated with azoospermia

In the univariate analysis, having undergone conditioning with TBI of 7.5 Gy or over ($P<0.0001$), ongoing chronic GVHD at time of last SFA ($P=0.004$), and age over 25 years at HSCT ($P=0.01$) were significant factors associated with azoospermia. When comparing the impact of the three main conditioning regimens on spermatogenesis, there was a clear statistical difference ($P<0.0001$; Figure 1). Factors such as acute GVHD ($P=0.051$), chronic GVHD ($P=0.063$), being still on immunosuppression at time of SFA ($P=0.058$) as well as having a time interval of less than eight years between HSCT and SFA ($P=0.063$) were of borderline significance (Table 3). Levels of FSH at time of SFA were significantly higher in azoospermic patients 14.9 (3.3-40.6) IU/l, compared to those with any degree of spermatogenesis 9.8 (2.0-25.7) IU/l ($P=0.007$). Levels of LH and testosterone were 7.6 (2.2-20.3) IU/l and 16.3 (3.4-373) ng/L in azoospermic patients *versus* 5.2 (1.8-14.3) IU/l and

Table 1. Patients' characteristics and HSCT features.

N. of allogeneic HSCT	224
Median age (years) at HSCT (range)	24 (2-59)
Number of patients	
≤25 years at HSCT	131 (58%)
>25 years at HSCT	93 (42%)
Source of stem cells	
Bone marrow	173 (77%)
PBPC	50 (22%)
CB	1 (0.5%)
Myeloablative conditioning	199 (93%)
Conditioning regimen	
with TBI (≥7.5 Gy)	146 (66%)
with busulfan	44 (20%)
no TBI (or 2Gy) and no busulfan	31 (14%)
Radiotherapy before HSCT	12 (6%)
infra-diaphragmatic	2 (1.5%)
Disease	
malignant	195 (87%)
non-malignant	29 (13%)
Donor type	
identical sibling	190 (85%)
syngeneic	3 (1%)
matched unrelated	22 (10%)
mismatched (relatives or unrelated)	8 (4%)
Calendar year of HSCT	
before 1990	48 (21%)
1990- 2000	97 (43%)
after 2000	79 (35%)
Acute GVHD	142/222 (64%)
Grade of acute GVHD	
no and grade I GVHD	139/217 (64%)
Grade II-IV	78/217(36%)
Chronic GVHD	134/220 (61%)
Extent of chronic GVHD	
no or limited chronic GVHD	153/204 (75%)
extensive	51/204 (25%)
Chronic GVHD at time of SFA	63/191 (33%)
On immunosuppression at time of SFA	52/202 (26%)

Table 2. Results at last SFA, gonadotropines and testosterone level of 224 patients.

N. of SFA with sperm results	224
N. of SFA/patient (after HSCT)	
one	166 (74%)
two or more	58 (26%)
N. of patients with an SFA before HSCT	23 (9%)
N. of patients with an SFA before and after HSCT	17 (8%)
Median age at last SFA (years)	31 (14-64)
Time between HSCT and last SFA (months/range)	63 (8 - 275)
Spermatogenesis according to WHO classification	
normozoospermia (>20x10^6/mL)	22 (10%)
oligozoospermia (5-20x10^6/mL)	13 (6%)
severe oligozoospermia(<5x10^6/mL)	28 (13%)
cryptospermia (sperm presence only after centrifugation)	7 (3%)
azoospermia (no sperm)	154 (69%)
Sperm concentration of SFA with sperms present	
n. of SFA with sperms	56
median concentration (x10^6/mL) (range)	5.6 (0-165)*
Sperm motility (in percentage)	
n. of SFA	58
median percentage of sperm motility (range)	50% (0-92%)
Sperm morphology	
n. of SFA	36
median percentage of normal morphology (%) (range)	4 (0-90)
FSH serum level	
n. of patients	80
level (median; range) (IU/L)	13.6 (2.0-40.6)
LH serum level	
n. of patients	80
level (median; range) (IU/L)	7.1 (1.8-20.3)
Testosterone serum level	
n. of patients	74
Level (median; range) (ng/dL)	15.0 (1.81-373.0)

**In case of cryptospermia.*

13.4 (1.81-268) ng/L in patients with sperm, respectively (P=0.091 and P=0.079). There is no difference in FSH at time of SFA between patients with and without ongoing chronic GVHD (P=0.708).

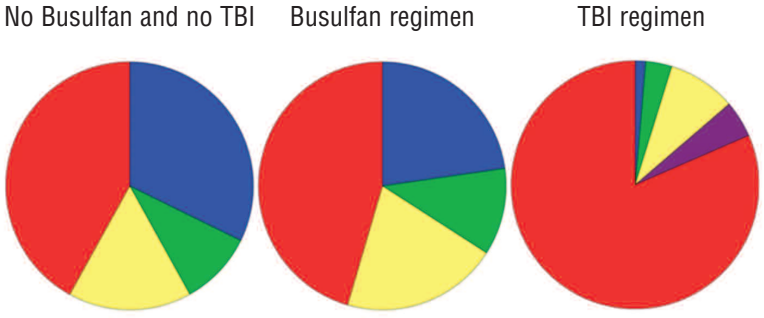
In multivariate backward stepwise logistical regression analysis, being conditioned with TBI (RR 7.1; 95% CI: 3.4-14.8) and age over 25 years at transplantation (RR 2.4; 95% CI: 1.09-5.2) were significantly associated with higher risk for azoospermia, whereas the presence of ongoing chronic GVHD at SFA showed a trend to remain azoospermic (Table 4). Since conditioning with TBI presented the strongest impact on the risk of infertility after HSCT, a multivariate analysis not including TBI as a variable was performed. Thus, ongoing chronic GVHD at SFA arises as the only significant risk factor (Table 4). In a subgroup analysis of patients conditioned without TBI, ongoing GVHD was the only risk factor, with an RR of 3.11 (95% CI: 1.02-9.47; P=0.045).

Risk score for azoospermia

Out of 224 patients, 188 were included in the risk score analysis. The risk score for azoospermia was highly predictive: 36% (10 of 28) of the long-term survivors with a low risk score (0-1), 67% (67 of 100) of the long-term survivors with an intermediate score (1.5-3), and 92% (55 of 60) of the long-term survivors with a high risk score (3.5-4.5) had azoospermia after allogeneic HSCT (P=0.0001) (Figure 2). In the high-risk score group there were no patients who fathered a child naturally.

Longitudinal sperm recovery analysis

Data from 58 patients were available for a longitudinal analysis of the sperm recovery. The median time interval between HSCT and first or last SFA was 49 months (range 1-269), and 87 months (range 28-275), respectively. The median time interval between first and last SFA was 24 months (range 1.5-140 months). At first SFA, 7 patients (12%) were normozoospermic, one (2%) was oligozoospermic, 4 (7%) severely oligozoospermic, 3 (5%) cryptospermic, and 43 (74%) azoospermic. At last SFA, 9 (15%) were normozoospermic, 3 (5%) oligozoospermic, 6 (10%) severely oligozoospermic, 4 (7%) cryptospermic, and 36 (62%) azoospermic (P>0.0001). In 12 (21%) out of 58 patients, there was an increase in sperm counts. We compared



Number of patients	31	44	146	Total
Normozoospermia	10(32%)	10(23%)	2(1%)	22
Oligozoospermia	3(32%)	5(11%)	5(4%)	13
Severely oligozoospermia	5(16%)	9(20%)	13(2%)	27
Cryptospermia	0(0%)	0(0%)	7(5%)	7
Azoospermia	13(42%)	20(46%)	119(81%)	152

Malignant disease	12(39%)	38(86%)	142(97%)	192
Non-malignant disease	19(61%)	6(14%)	4(3%)	29

Figure 1. Relationship between type of conditioning (TBI containing regimen; busulfan containing regimen, and regimen without TBI and busulfan) and type of disease (malignant *vs.* non-malignant) compared to the sperm content in the seminal fluid analysis. The majority of patients with malignant diseases were conditioned with a TBI containing regimen.

Table 3. Univariate analysis. Risk factors associated with azoospermia.

Factor	N.	Azoospermic	Evidence of spermatogenesis	*P*
TBI (≥7.5 Gy)	146	119 (82%)	27 (18%)	**<0.0001**
Acute GVHD	142	104 (73%)	38 (27%)	0.051
Chronic GVHD	134	99 (74%)	35 (26%)	0.063
Extensive chronic GVHD	51	41 (80%)	10 (20%)	0.062
cGVHD at time of last SFA	63	52 (83%)	11 (17%)	**0.004**
On IS at time of SFA	52	41 (79%)	11 (21%)	0.058
> 25 years of age at HSCT	87	69 (79%)	18 (21%)	**0.010**
< 8 years time interval	148	109 (74%)	39 (26%)	0.063

Table 4. Multivariate backwards logistical regression analysis including first all patients. In a second step of the analysis TBI as variable was excluded.

All patients	RR	95% CI	*P*
TBI present	7.130	3.415-14.887	<0.0001
Age >25 years at HSCT	2.406	1.094-5.292	0.029
Chronic GVHD at time of SFA present	2.178	0.950-4.993	0.066
All patients excluding TBI as a variable			
Chronic GVHD at time of SFA present	2.576	1.221-5.434	0.013

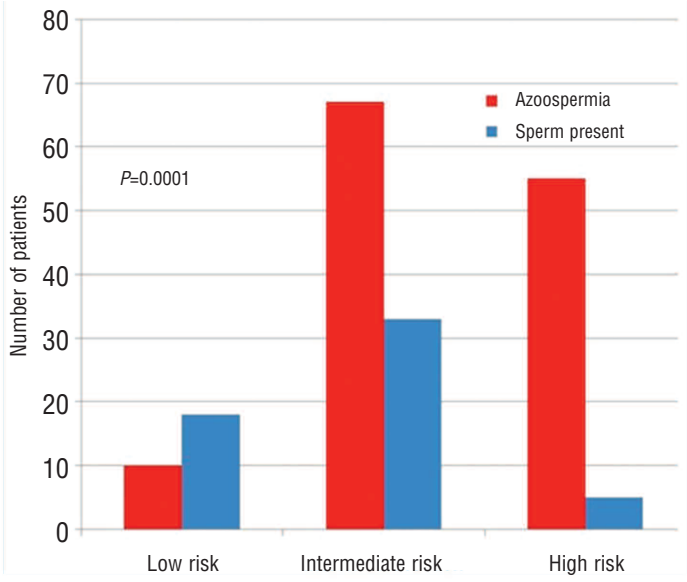
patients with sperm recovery between first and last SFA after HSCT to those without recovery. Patients with sperm recovery had significant longer follow up between first and last SFA (31 months; range 15-140), as compared to those without recovery (19 months; range 1.5-100; *P*=0.015) (*Online Supplementary Table S1*). In contrast, age at HSCT, TBI for conditioning, acute and chronic GVHD did not influence the recovery during follow up. We further compared the sperm concentration at first and last SFA. Using the non-parametric signed-rank test comparing two related samples, sperm concentration at first SFA was significantly lower compared to the concentration at last SFA, with median 5.01 (range 0-101) and 10.6 (range 0-165)x10^6/mL, respectively (*P*=0.019) (*Online Supplementary Figure S1*).

Analysis of patients with sperm analysis before and after HSCT

In 28 patients, seminal fluid had been collected before HSCT and was available for the analysis. Sperm could be detected in 27 (96%): 20 (71%) of them presented normozoospermia, 4 (14%) oligozoospermia, 3 (11%) severe oligozoospermia, and one (4%) azoospermia. All patients with decreased sperm counts in the pre-transplant SFA had malignant disease (AML n=3; CML n=2; lymphoma n=2; MDS/MPN n=1), and most of them were heavily pretreated. Data from 17 of these 28 patients were also available post-transplant. After HSCT, 14 of 17 patients (82%) had azoospermia (10 of them conditioned with TBI), one oligozoospermia and 2 severe oligozoospermia.

Paternity

After HSCT, 29 of 211 (14%) patients with a median age at HSCT of 21 (2-55) years, became fathers (total number of children 44). The time interval between HSCT and the birth of the first child was a median of 7.2 (1-21.6) years. Among the patients who fathered a child after HSCT, 11 fathered naturally, and 11 were assisted conceptions (cryopreserved sperms); there is no information on conception for 7 patients. Among the 11 patients who fathered a child naturally, none was in the group of high-risk score.



Score	0-1	1.5-3	3.5-4.5
Number of patients	28	100	60
Sperm present	18	33	5
Azoospermia	10	67	55
Percentage with azoospermia	36%	67%	92%

Figure 2. Risk of azoospermia according to score. TBI counted for 2 points, age over 25 years at HSCT and ongoing chronic GVHD at time of SFA for 1 point, and time interval between HSCT and SFA shorter than eight years for 0.5 point.

Discussion

To our knowledge, this is the largest cohort of male patients in which spermatogenesis has been evaluated following allogeneic HSCT. In this study, one-third of all patients had some degree of spermatogenesis post-transplant, and 10% of patients had a normal sperm count. As previously reported,[7,9,10,13,14,16,17] TBI was the strongest factor predicting infertility. However, TBI was not invariably linked to azoospermia since 18% of all patients conditioned with TBI had residual and/or recovered sperm production. On the other hand, patients not receiving TBI may also be azoospermic post-HSCT (Figure 1).

Patients transplanted with a busulfan containing conditioning regimen have some degree of spermatogenesis post-transplant, as well as a real chance to become father of a child.[9,18] We have shown that the risk of azoospermia after busulfan is not significantly higher than for patients conditioned with regimens excluding both TBI and busulfan. There are little data showing a relationship between sperm recovery and GVHD although in previous single center studies such a relationship has been suspected.[13,16,18] Here we have demonstrated that independent of TBI, age over 25 years at HSCT and ongoing chronic GVHD can be responsible for infertility after HSCT. However, the mechanism by which GVHD may lead to azoospermia remains unclear. In an animal model, injury to Leydig cells correlates with an intratesticular inflammatory response. Alloreactive donor T cells have been shown to infiltrate the testis during acute GVHD resulting in an impairment of testosterone-producing cells. This experiment did not show any evidence of a direct T-cell infiltration of seminiferous tubules, but rather speaks for an indirect effect of the GVHD on spermatogenesis, leading to a loss of Leydig cell function.[19] We cannot exclude the possibility that systemic inflammatory factors of ongoing chronic GVHD could play a role in sperm production. Nevertheless, GVHD did not affect FSH secretion.

To accurately interpret the impact of treatment on post-transplant infertility, we need to know the degree of gonadal dysfunction before HSCT. Decreased sperm concentration before HSCT can be due to the underlying disease itself. Indeed, oligozoospermia has been reported in 25% of patients with Hodgkin's lymphoma and in 57% of patients with leukemia before starting any treatment.[20] Decreased sperm concentration can also be the consequence of treatment-induced gonadal dysfunction before HSCT.[21-24] The most harmful drugs are nitrogen mustard derivates and alkylating agents.[25] In a subgroup analysis we showed that 71% of the patients were normozoospermic before HSCT. Therefore, in our cohort, the main reason for decreased spermatogenesis after HSCT was directly related to the transplantation procedure itself.

This high proportion of normozoospermic patients observed here before transplantation could be an overestimation. Indeed, sperm concentration does not decrease immediately after cytotoxic treatment. During the first 1-2 months sperm counts may remain normal and then diminish later during treatment. After radiation with a dose between 0.35 and 0.5 Gy, the nadir of sperm count occurs after 4-6 months only. Spermatogenic stem cells are more sensitive to chemotherapy and radiation, while later stage germ cells continue to mature after chemotherapy and radiation therapy. Therefore, recovery of spermatogenesis depends on the degree of destruction of the early sperm stem cells.[26]

There are few data on longitudinal recovery of fertility

after HSCT. Following cyclophosphamide and TBI conditioning,[16] or conditioning with carmustine, etoposide, cytosine arabinoside and melphalan (BEAM),[7] only anecdotal reports of recoveries have been made. Recovery of testicular function defined by normal LH, FSH and testosterone levels have been described[27] mainly in severe aplastic anemia patients.[28] This is not surprising, since patients with aplastic anemia do not require chemotherapy before HSCT, and are usually conditioned with a reduced intensity regimen. In a subgroup analysis, we show that sperm recovery is possible, and is more likely the longer the time interval between HSCT and SFA.

With a new scoring system, based on the four predictors for decreased sperm production, which are TBI, age over 25 years old at HSCT, ongoing chronic GVHD, and time interval between HSCT and SFA under eight years, we were able to detect long-term survivors with a probability of more than 90% of being azoospermic. This risk score does not replace the SFA for fertility assessment after HSCT, but can be used as a tool for patient counseling. The findings of this study have direct repercussions on long-term male survivors and their respective partners. These results may also support health care providers in counseling patients before and after HSCT. Because of the high probability of infertility after HSCT, fertility preservation by cryopreservation of spermatozoa early during the course of the disease before HSCT has to be advised.[29,30] However, since late recovery of fertility is possible, contraception should be recommended and periodical SFA, particularly in patients with a low-risk score for infertility, should be considered.

Our study has some limitations. First, this is a retrospective analysis with SFA performed at different centers, at different time points after HSCT, and for various reasons. The centers reported only on patients for whom an SFA had been performed. We have data on hormonal status such as FSH[10,31,32] LH and testosterone levels, but not on inhibin B, a parameter that seems to be more closely related to sperm activity.[33-35]

In conclusion, TBI is still the most relevant adverse risk factor for sperm production after HSCT. However, age over 25 years at HSCT, ongoing chronic GVHD, and short follow up after transplantation are also relevant obstacles to sperm recovery. In patients not conditioned with TBI, ongoing chronic GVHD is the main adverse factor for sperm recovery. Therefore, for the first time, evidence for a graft-*versus*-testis effect can be demonstrated to be the most relevant adverse factor for sperm recovery in patients conditioned without TBI. An improved understanding of the risk factors involved in gonadal dysfunction and the potential return to fertility will help improve counseling for patients undergoing HSCT and has direct implications for the quality of life of long-term survivors.

Acknowledgments

We thank Prof. Alois Gratwohl for his continued encouragement and support in this study. We would also like to thank Dr Zeynep Aki, Sylvie Lengay, Rosi Oneto, Jaana Vettenranta, Emanuela Marrocco, Ed Colcol, Maria Waeltermann, Gülsün Tezcan, Rik Lewin and Ann Devos for their valuable contribution to improving the data collection.

Appendix of EBMT participating centers (CIC, country, city, investigator/s, number of patients)

169, Turkey, Ankara, Gülsan Sucak, 15; 202, Switzerland, Basel, Alicia Rovó, 39; 205, United Kingdom, London (Imperial College), Nina Salooja, 30; 207, France, Paris (Saint-Louis), Gérard Socié, 1; 213, France, Paris (Saint-Antoine), N C Gorin, 1; 217, Italy, Genoa, Sandra Chiodi, Simonetta Spinelli, Maria Teresa Van Lint, 64; 219, Finland, Helsinki, Kirsi Jahnukainen, 6; 279, Italy, Monza, Cornelio Uderzo, 5; 285, Italy, Padua, Marta Pillon, 1; 305, Italy, Torino, Eleonora Biasin, 1; 397, Saudi Arabia, Riyadh, Mahmoud Aljurf, 51; 505, Germany, Munster, Karoline Ehlert, 6; 556, Hungary, Budapest, Tamás Masszi, 2; 608, United Kingdom, Swindon, Norbert Blesing, 1; 609, Malaysia, Kuala Lumpur, Tan Swee, 8; 618, Turkey, Antalya, Kim Akif Yesilipek, 6; 713, United Kingdom, Leicester, Ann Hunter, 12; 859, Bulgaria, Sofia, Penka Ganeva, 2; 996, Belgium, Antwerp, Wilfried Schroyens, 3.

Authorship and Disclosures

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

References

1. Copelan EA. Hematopoietic stem-cell transplantation. N Engl J Med. 2006;354(17):1813-26.
2. Baldomero H, Gratwohl M, Gratwohl A, Tichelli A, Niederwieser D, Madrigal A, et al. The EBMT activity survey 2009: trends over the past 5 years. Bone Marrow Transplant. 2011;46(4):485-501.
3. Gratwohl A, Baldomero H, Schwendener A, Gratwohl M, Apperley J, Frauendorfer K, et al. The EBMT activity survey 2008: impact of team size, team density and new trends. Bone Marrow Transplant. 2011; 46(2):174-91.
4. Bevans MF, Mitchell SA, Barrett AJ, Bishop M, Childs R, Fowler D, et al. Function, Adjustment, Quality of Life and Symptoms (FAQS) in Allogeneic Hematopoietic Stem Cell Transplantation (HSCT) Survivors: A Study Protocol. Health Qual Life Outcomes. 2011;9(1):24.
5. Wong FL, Francisco L, Togawa K, Bosworth A, Gonzales M, Hanby C, et al. Long-term recovery after hematopoietic cell transplantation: predictors of quality-of-life concerns. Blood. 2010;115(12):2508-19.
6. Mayer DK, Ratichek S, Berhe H, Stewart S, McTavish F, Gustafson D, et al. Development of a health-related website for parents of children receiving hematopoietic stem cell transplant: HSCT-CHESS. J Cancer Surviv. 2010;4(1):67-73.
7. Jacob A, Barker H, Goodman A, Holmes J. Recovery of spermatogenesis following bone marrow transplantation. Bone Marrow Transplant. 1998;22(3):277-9.
8. Mertens AC, Ramsay NK, Kouris S, Neglia JP. Patterns of gonadal dysfunction following bone marrow transplantation. Bone Marrow Transplant. 1998;22(4):345-50.
9. Sanders JE, Hawley J, Levy W, Gooley T, Buckner CD, Deeg HJ, et al. Pregnancies following high-dose cyclophosphamide with or without high-dose busulfan or total-body irradiation and bone marrow transplantation. Blood. 1996;87(7):3045-52.
10. Borgmann-Staudt A, Rendtorff R, Reinmuth S, Hohmann C, Keil T, Schuster FR, et al. Fertility after allogeneic haematopoietic stem cell transplantation in childhood and adolescence. Bone Marrow Transplant. 2012;47(2):271-6
11. Hammond C, Abrams JR, Syrjala KL. Fertility and risk factors for elevated infertility concern in 10-year hematopoietic cell transplant survivors and case-matched controls. J Clin Oncol. 2007;25(23):3511-7.
12. Chatterjee R, Goldstone AH. Gonadal damage and effects on fertility in adult patients with haematological malignancy undergoing stem cell transplantation. Bone Marrow Transplant. 1996;17(1):5-11.
13. Rovo A, Tichelli A, Passweg JR, Heim D, Meyer-Monard S, Holzgreve W, et al. Spermatogenesis in long-term survivors after allogeneic hematopoietic stem cell transplantation is associated with age, time interval since transplantation, and apparently absence of chronic GvHD. Blood. 2006;108(3):1100-5.
14. Savani BN, Kozanas E, Shenoy A, Barrett AJ. Recovery of spermatogenesis after total-body

irradiation. Blood. 2006;108 (13):4292-3.
15. [Laboratory manual of the WHO for the examination of human semen and sperm-cervical mucus interaction]. Ann Ist Super Sanità. 2001;37(1):I-123.
16. Anserini P, Chiodi S, Spinelli S, Costa M, Conte N, Copello F, et al. Semen analysis following allogeneic bone marrow transplantation. Additional data for evidence-based counselling. Bone Marrow Transplant. 2002;30(7):447-51.
17. Pakkala S, Lukka M, Helminen P, Koskimies S, Ruutu T. Paternity after bone marrow transplantation following conditioning with total body irradiation. Bone Marrow Transplant. 1994;13(4):489-90.
18. Grigg AP, McLachlan R, Zaja J, Szer J. Reproductive status in long-term bone marrow transplant survivors receiving busulfan-cyclophosphamide (120 mg/kg). Bone Marrow Transplant. 2000;26(10):1089-95.
19. Wagner AM, Beier K, Christen E, Hollander GA, Krenger W. Leydig cell injury as a consequence of an acute graft-versus-host reaction. Blood. 2005;105(7):2988-90.
20. Chung K, Irani J, Knee G, Efymow B, Blasco L, Patrizio P. Sperm cryopreservation for male patients with cancer: an epidemiological analysis at the University of Pennsylvania. Eur J Obstet Gynecol Reprod Biol. 2004;113(Suppl 1):S7-11.
21. Howell S, Shalet S. Gonadal damage from chemotherapy and radiotherapy. Endocrinol Metab Clin North Am. 1998; 27(4):927-43.
22. Howell SJ, Shalet SM. Testicular function following chemotherapy. Hum Reprod Update. 2001;7(4):363-9.
23. Sklar CA, Kim TH, Ramsay NK. Testicular function following bone marrow transplantation performed during or after puberty. Cancer. 1984;53(7):1498-501.
24. Howell SJ, Shalet SM. Effect of cancer therapy on pituitary-testicular axis. Int J Androl. 2002;25(5):269-76.
25. Dohle GR. Male infertility in cancer patients: Review of the literature. Int J Urol. 2010;17(4):327-31.
26. Trottmann M, Becker AJ, Stadler T, Straub J, Soljanik I, Schlenker B, et al. Semen quality in men with malignant diseases before and after therapy and the role of cryopreservation. Eur Urol. 2007;52(2):355-67.
27. Tauchmanova L, Selleri C, Rosa GD, Pagano L, Orio F, Lombardi G, et al. High prevalence of endocrine dysfunction in long-term survivors after allogeneic bone marrow transplantation for hematologic diseases. Cancer. 2002;95(5):1076-84.
28. Sanders JE, Buckner CD, Leonard JM, Sullivan KM, Witherspoon RP, Deeg HJ, et al. Late effects on gonadal function of cyclophosphamide, total-body irradiation, and marrow transplantation. Transplantation. 1983;36(3):252-5.
29. Schover LR, Brey K, Lichtin A, Lipshultz LI, Jeha S. Oncologists' attitudes and practices regarding banking sperm before cancer treatment. J Clin Oncol. 2002;20(7):1890-7.
30. Schover LR, Brey K, Lichtin A, Lipshultz LI, Jeha S. Knowledge and experience regarding cancer, infertility, and sperm banking in younger male survivors. J Clin Oncol. 2002;20(7):1880-9.
31. Brennemann W, Stoffel-Wagner B, Wichers M, Helmers A, Albers P, Mezger J, et al. Pretreatment follicle-stimulating hormone: a prognostic serum marker of spermatogenesis status in patients treated for germ cell cancer. J Urol. 1998;159(6):1942-6.
32. Thomson AB, Anderson RA, Irvine DS, Kelnar CJ, Sharpe RM, Wallace WH. Investigation of suppression of the hypothalamic-pituitary-gonadal axis to restore spermatogenesis in azoospermic men treated for childhood cancer. Hum Reprod. 2002;17(7):1715-23.
33. Brennemann W, Stoffel-Wagner B, Bidlingmaier F, Klingmuller D. Immunoreactive plasma inhibin levels in men after polyvalent chemotherapy of germinal cell cancer. Acta Endocrinol. 1992; 126(3):224-8.
34. Brennemann W, Sommer L, Stoffel-Wagner B, Bidlingmaier F, Klingmuller D. Secretion pattern of immunoreactive inhibin in men. Eur J Endocrinol. 1994;131(3):273-9.
35. Wallace EM, Groome NP, Riley SC, Parker AC, Wu FC. Effects of chemotherapy-induced testicular damage on inhibin, gonadotropin, and testosterone secretion: a prospective longitudinal study. J Clin Endocrinol Metab. 1997;82(9):3111-5.